MIST: Microscopy Image Stitching Tool

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***Abstract*—Automated microscopy enables scientists to image an area of an experimental sample that is much larger than the microscope's Field of View (FOV) and to carry out time-lapse studies of cell cultures. We address the problem of creating image mosaics from a grid of overlapping tiles constrained to only translational offsets.**

This work describes a stitching method called MIST (Microscopy Image Stitching Tool) with minimized translational uncertainty for large collections of grid-based microscopy tiles. The method improves tile translations computed using a registration method, such as the Fourier transform based phase correlation, by optimizing the normalized cross correlation between the overlap of adjacent tiles. The optimization incorporates mechanical properties of a microscope stage to improve all translations using constrained Hill Climbing restricted to a searching square area of 4x the stage repeatability per side.

We also present a methodology to quantify stitching accuracy by creating a reference dataset from one's own experiment and choose the best performing stitching method accordingly. The stitching accuracy is evaluated by comparing the reference measurements to the stitched measurements. The measurements are centroid and area of regions of interest (ROI) that fits within a Field of View (FOV). The quantification is done using four NIST-derived metrics: false positive (added ROIs), false negative (undetected ROI), centroid distance error and area error.

***Index Terms*— stitching**